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Tolerance of N-Chlorotaurine, an Endogenous Antimicrobial Agent, in the Rabbit and Human Eye— A Phase I Clinical Study

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ABSTRACT

N-chlorotaurine (NCT), an essential weak oxidative N-chloro compound produced by stimulated human leukocytes, shows bactericidal, fungicidal, virucidal and vermicidal efficacy. A double-blind, randomized and placebo controlled study was done to evaluate the tolerance of the aqueous NCT solution by application to rabbit and human conjunctiva. In six rabbits treated with 1% and 3% NCT regimen for nine days no ocular and behaviour changes could be observed. In a pilot study with two volunteers, treatment with 2.8% NCT for five days caused a self-limited conjunctival injection of one subject, while 1% NCT was well tolerated. Subsequently, eight healthy volunteers participated in a phase I clinical study. One percent NCT was applied for five days and was well tolerated by all subjects except for minimal eye burning after the application. Because of these positive results, usage of the antimicrobial agent NCT in ophthalmology is suggested.

INTRODUCTION

N-chlorotaurine (Cl-HN-CH_2-CH_2-SO_3H, NCT), the N-chloro derivative of the amino acid taurine, is an essential weak oxidant produced by human granulocytes and macrophages during inflammatory reactions (1-4). It is thought to play a role in destruction of pathogens (5).

Recently, it was possible to make available NCT in the form of the crystalline sodium salt which induced a large scale *in vitro* investigation of its microbicidal properties (6). NCT demonstrated significant microbicidal activity against bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*), yeasts (*Candida albicans*) and viruses (*Herpesvirus types 1* and *2, Adenovirus type 5*)(7-9). Additionally, its vermicidal effect on *Schistosoma mansoni* and the activation of complement C5 *in vitro* has been confirmed (10,11).

On the other hand, cytotoxicity of NCT against leukocytes and erythrocytes (1), lung epithelial cells (12,13) and murine macrophages RAW 264.7 (14) proved to be low compared to the more powerful oxidant, hypochlorite. Moreover, hypochlorite and monochloramine, but not NCT, increased

microvascular permeability in isolated perfused rat lungs (15). Other data suggest that NCT may minimize damage to cartilaginous joint structures in inflammatory arthritis by inactivating collagenase (16).

Because of low cytotoxicity and significant antimicrobial activity, NCT is potentially of use in the treatment of infective conjunctivitis. The aim of this study was to investigate the tolerability of NCT in rabbit and human conjunctiva.

MATERIALS AND METHODS

Materials

NCT was prepared as the crystalline sodium salt (MW = 181.52 g/mol) (6) and its purity verified by iodometric titration (calculated 19.53% Cl^+, found 19.3 % Cl^+, which equals 99% purity). Solutions of NCT in sterile distilled water at concentrations of 1.0%, 2.8% and 3.0% (55, 154 and 165 mM, respectively) were dispensed into 8 ml pipette-tubes. Control tubes were filled with sterile 0.9% NaCl. Since aqueous solutions of NCT exhibit a pH ≈ 8 and, moreover, a broad spectrum of activity against pathogens (7-10), no preservatives or buffers, respectively, were felt to be necessary. Stock solutions stored at 2-4°C showed high stability (9.3 percent loss per year, determined photometrically at 252 nm (1,8)).

Study Design

Animal Subjects

The animal experiments were approved by the Austrian Bundesministerium für Wissenschaft und Forschung and followed the "Principles of laboratory animal care". Six rabbits (from SAVO, Kissleg/ FRG) were randomized and divided in two groups: one eye of three animals was treated with 1% NCT, the randomized control eye with 0.9% NaCl. The other three rabbits were treated the same way but with 3% NCT. One drop of study drug was applied to the conjunctiva hourly eight times a day during the first two days of the experiment and two-hourly five times a day during the next seven days. Applications and examinations were performed in a blind fashion. The external parts of the eye (conjunctiva, cornea, sclera, iris) were examined daily by usage of a slit lamp and magnifying glasses. The grading pattern of adverse effects was similar to that used in human subjects described below.

Additionally, effects on food intake and movement were judged. Further examinations were performed at the end of the treatment and seven months later to exclude long-time adverse effects.

Human Subjects

The Ethics Committee of the University of Innsbruck approved the study, which was performed in accordance with the Declaration of Helsinki. All subjects gave written informed consent. Health status was determined by evaluation of medical history, by physical examination and, especially, by a detailed ophthalmological examination (see below).

Both a previous pilot study and the main study were designed as a double-blind, randomized, placebo-controlled clinical phase I trial. One eye of each subject received NCT and one placebo (0.9% NaCl), assigned in a randomized fashion, one drop each as a single dose.

Pilot Study

A pilot study with two male, 27 and 59 year old subjects was done to identify the tolerated dose and to get pharmacodynamic data to identify the optimal time interval between drops of NCT. One percent

and, one week later, 2.8% NCT (of physiological osmolality) versus placebo was investigated using five daily applications at an interval of two hours for five days each. Both subjects were examined daily subsequent to the fifth single dose as described below.

For qualitative examination of pharmacodynamics, lacrimal fluid was collected 5, 10, 15 and 20 minutes subsequent to application of 1% NCT using a strip of filter paper (Schleicher & Schuell, width of five millimeters) placed onto the conjunctiva bulbi and palpebrae inferioris for five seconds. One drop each of potassium iodide and starch solutions were applied onto the wet strip which caused the formation of blue color (17) in the presence of NCT ($NCT + 2I^- + H^+$ -----> taurine + I_2 + Cl^-).

Phase I Study

Eight volunteers (five male, three female, ranging in age from 24 to 59 years, mean 38 years, standard deviation 14.4) participated and were treated for five days. Since NCT in the conjunctival fluid could be detected for 15 minutes after dosing in the pilot study (see results), during the following phase I trial five serial single doses at an interval of 15 minutes were applied once daily to ensure a level of oxidative action for one hour. All subjects were examined daily subsequent to each fifth single dose during the treatment cycle as well as six (all subjects), thirteen (three subjects), 33 (four subjects) and 41 days later (two subjects) by usage of a slit lamp and an ophthalmoscope. The first examination was done prior to participation. The following parts of the eye were examined: conjunctiva, caruncula, cornea, sclera, glandula lacrimalis, iris, lens, choroidea and retina. Subjective adverse effects included criteria, such as pain (eye burning, headache, pain of the eyelid), impaired vision (unsharp vision, photophobia, colored vision, impaired visual field) and other impairments as well as their intensity, duration and time relationship to the application of NCT/placebo. Adverse effects were graded into: i) *minimal* - hardly noticeable subjective effects and objective ones like discrete vascular injection and edema of the above mentioned examined regions; ii) *medium* - subjective effects with impairment of condition, pronounced objective effects, but self-limited and not threatening health; and iii) *strong* - sudden intense subjective effects, objective effects threatening health or requiring therapeutic intervention.

Statistical Analysis

Adverse effects were evaluated by descriptive statistical analysis (frequencies, minimum, maximum and median). Frequency of each side effect was calculated in relation to the number of subjects as well as to the number of days of treatment. Additionally, Pearson's Chi-Square test was used to test for statistical significance, and P values of less than 0.05 were considered significant.

RESULTS

Tolerability of NCT in the Rabbit Eye

During the treatment with 1% and 3% NCT, as well as seven months later, no ocular alterations could be detected. There were no changes in the behaviour of the animals.

Tolerability of NCT in the Human Eye

Pilot Study and Pharmacodynamics of NCT

In the pilot study with two volunteers, 1% NCT was as well tolerated as 0.9% NaCl by both subjects without occurrence of adverse effects. NCT 2.8%, however, caused conjunctival irritation of one subject after two days, specifically medium graded conjunctival injection and edema of the nasal conjunctiva bulbi, so that premature ending of treatment was necessary. Nevertheless, these disorders disappeared

within two days without necessity of any additional therapy. The second volunteer completed five days of study therapy with 3% NCT without occurrence of adverse effects.

Oxidative activity was detectable for fifteen minutes after application of one drop of 1% NCT in both subjects.

Phase I Study

In this study, seven of eight subjects remarked eye burning, eye itching and irritation of a few minutes to two hours duration subsequent to the application of the five single doses of NCT but not NaCl. These adverse effects, however, were of such small magnitude (graded minimal, similar to the entry of sweat to the conjunctiva) that no subject required premature treatment discontinuation. Impaired vision and other impairments were not noted.

Objectively, minimal conjunctival (pinguecula inflammata, edema), episcleral (injection, edema) and caruncular (tumefaction) irritations could be observed in both the NCT and placebo group. No alteration of the cornea could be detected. Chronological appearance and overlaps of adverse effects in each subject are shown in table 1. Both subjective and objective events not only appeared but also disappeared again despite continued treatment. Increased lacrimation of one subject found at the second day disappeared again after a few hours. There was no accumulation of adverse effects at single days of the therapy cycle and no overlap of episcleral and conjunctival events. The most frequent overlapping adverse effect was eye burning.

The number of subjects that experienced an event as well as the frequency of events and statistical analyses are summarized in table 2. Only the frequency of eye burning was statistically significant.

No adverse effects requiring premature study discontinuation occurred, and no alterations of the middle and internal parts of the bulbus oculi were noted. No long-term adverse effects appeared during follow-up.

DISCUSSION

Previous *in vitro* investigations suggest low degree of cytotoxicity of *micro*-molar NCT against cells and tissue (1,12-16). This study for the first time demonstrated good tolerability of a *milli*-molar concentration of NCT after application to the eye. One percent (55 mM) NCT was well tolerated with minimal subjective adverse effects that were expected because of the oxidative activity. In the objective category, absolute frequency of events in the NCT group was higher, but this difference was not significant. Episcleral and conjunctival vascular injection were of such small magnitude that it was difficult to distinguish them from daily fluctuations in vascularization of the healthy eye. A reasonable safety margin was demonstrated for 1% NCT, as the high concentration of 2.8% NCT showed no more complications than a harmless, self-limited conjunctival injection. NCT is an endogenous substance (N-chloro derivative of an amino acid) so that no allergic reactions are likely to occur.

NCT 1% has demonstrated significant microbicidal properties in buffer solution as well as in human inflammation samples within incubation times of a few to sixty minutes (7-9). Therefore this concentration seems to be the optimal one with regard to both efficacy and tolerability. Nevertheless, for sufficient killing of bacteria, yeasts and viruses, a 15- minutes duration of action after one dose might be too short for practical use. Application of five serial single doses at 15- minute intervals seems to be superior to longer intervals, as oxidative efficacy will be provided for more than one hour.

Until now, there has been no literature supportive of the application of an active chlorine compound in ophthalmology to treat infections. This may be because the main representatives of this class of compounds, such as hypochlorites and chloramine T, which have been used for a long time in human medicine have been considered to be too reactive for this purpose. The present study supports evaluation of the active chlorine compound NCT in treatment of conjunctival infections, based upon tolerability in the human eye and broad spectrum of microbicidal activity.

TABLE 1

Chronological Appearance and Overlaps of Adverse Effects During the Five-day Treatment with NCT

		NCT-eye					Placebo-eye				
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
Subject 1	subjective	-	-	-	-	burning	-	-	-	-	-
	objective	episcleral	episcleral	episcleral	caruncular	episcleral	-	episcleral	-	caruncular	-
Subject 2	subjective	burning	burning	burning	-	itching	-	-	-	-	-
	objective	-	episcleral, lacrimation	-	-	-	-	-	-	-	-
Subject 3	subjective	burning	burning	-	irritation	-	-	-	-	-	-
	objective	-	-	-	-	-	-	-	-	-	conjunctival
Subject 4	subjective	itching	-	-	itching	burn..itching	-	-	-	-	-
	objective	-	-	-	-	conjunctival, caruncular	-	conjunctival	episcleral	-	-
Subject 5	subjective	-	-	burning	burning	burning	-	-	-	-	-
	objective	-	-	-	-	-	-	-	-	-	-
Subject 6	subjective	burning	burning	burning	burning	burning	-	-	-	-	-
	objective	caruncular	conjunctival	-	-	-	-	conjunctival	-	-	-
Subject 7	subjective	-	-	irritation	irritation	burning	-	-	-	-	-
	objective	-	-	-	-	-	-	-	-	-	-
Subject 8	subjective	-	-	-	-	-	-	-	-	-	-
	objective	-	-	-	-	-	-	-	-	-	-

TABLE 2

Statistical Analysis of Adverse Effects Illustrated in Table 1

	NCT-eye		Placebo-eye		Pearson's Chi-square
Adverse effect	Number of subjects * with adverse effects	Frequency of adverse effects per subject ** min - max ; median	Number of subjects * with adverse effects	Frequency of adverse effects per subject ** min - max ; median	p-value
a) Subjective					
eye burning	7	1 - 5 ; 2	0	0	< 0.01
eye itching	2	1 - 3 ; 2	0	0	0.45
irritation	2	1 - 2 ; 1.5	0	0	0.20
altogether subjective	7	1 - 5 ; 2	0	0	< 0.01
b) Objective					
episcleral (injection, edema)	2	1 - 4 ; 2.5	2	1 ; 1	0.57
conjunctival (edema, pinguecula inflammata)	2	1 ; 1	3	1 ; 1	1.00
caruncular tumefaction	3	1 ; 1	1	1 ; 1	0.57
increased lacrimation	1	1 ; 1	0	0	1.00
altogether objective	4	1 - 4 ; 1	4	1 ; 1	0.62

* total number of 8 subjects

** related to the number of five days of treatment of each subject

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